Effects of Fluoride on Pollen Germination, Pollen Tube Growth, and Fruit Development in Tomato and Cucumber

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ABSTRACT

Tomato and cucumber pollen germination in vitro was significantly inhibited at 10.5 and 2.6 mM NaF, respectively, or higher, in media containing 1.25 mM Ca. Germination was not inhibited provided the Ca was at least chemically equivalent to the F^- in the media. Long-term, continuous HF fumigations of tomato plants at 7.9 and 13.0 $\mu g\ F/m^3$ reduced pollen germination in vitro when the plants were grown with low Ca (1 mM) nutrient solution. No reduction was found at 13.0 $\mu g\ F/m^3$ when tomato plants were grown with 4 mM Ca. Regardless of Ca nutrition, HF fumigations of cucumber plants at 10.2 $\mu g\ F/m^3$ or less did not inhibit pollen germination. Fluorescent staining of pistils from manually pollinated tomato flowers showed reductions in the number of pollen grains retained on the stigma, pollen germination, and pollen tubes reaching ovules when the maternal parent plants were grown in HF treatments averaging as low as 4.2 $\mu g\ F/m^3$. Similar effects were found when pollen parents grown with 1 mM Ca were subjected to 7.9 $\mu g\ F/m^3$, but not when pollen parents grown with 5 mM Ca were subjected to 4.4 $\mu g\ F/m^3$. Early tomato fruit and seed development were inhibited by treatments similar to those that caused the pollen responses.

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Fluoride pollutants in the atmosphere apparently can affect seed and fruit production. Tomato plants grown in chambers under rather severe HF treatments produced smaller fruit than control plants, and the fruit were partially or completely seedless (7). Tomato plants grown with suboptimum levels of Ca showed similar effects on fruiting, and the effects of HF and low Ca nutrition were additive. Bean plants

grown under long-term, continuous exposure to fairly high HF levels produced fewer seeds per fruit and fewer fruit than control plants (8). These findings suggest that fluoride (F^-) may inhibit some aspect of fertilization or seed development, possibly by interfering with Ca metabolism.

The study reported here was conducted to investigate the effects of F^- on pollen germination, pollen tube growth, and early seed and fruit development, and to evaluate the modifying influence of Ca on these effects. Cucumber and tomato pollen germination was tested in vitro to evaluate (i) the effects of different concentrations of F^- and Ca in the germination media; and (ii) the effects of HF fumigation of plants growing in standard and low Ca nutrient solutions. Fluorescent microscopy was used to investigate the effects of HF on pollen germination and subsequent growth in situ in the tomato pistil.

MATERIALS AND METHODS.—All plants were grown and exposed to HF in plant growth chambers designed for air pollution research (1). All incoming air was cleaned to remove possible contaminants, and HF was introduced into the air stream entering one chamber by the method of Hill et al. (4). Other chambers receiving the cleaned air were used for control atmosphere treatments and for experiments that did not include HF treatments. Air from the HF-fumigation chamber was aspirated continuously through 0.01 N NaOH. The solution was changed twice daily and analyzed for F^- by titration with thorium nitrate using alizarin indicator (2). The F^- content of the control chamber atmosphere was determined by sampling for 2- to 4-week periods with glass fiber filters, isolating the F^- by perchloric acid distillation, and titrating with thorium nitrate (9).

Day-lengths of 14 to 16 hr were used, and the light intensity was about 1,500 ft-c at bench level. The temperature and relative humidity averaged 27 C and 40%, respectively, during the day and 17 C and 50% at night.

Tomato (*Lycopersicon esculentum* Mill. 'Michigan State Forcing') and cucumber (*Cucumis sativus* L. 'Marketer') plants were grown from seed in sand culture. The cucumbers were seeded in 1-gal stoneware pots and thinned to one plant/pot. Single tomato plants were transplanted to 1-gal pots when 10 to 15 cm tall. The plants were automatically subirrigated 3 times daily from reservoirs located in each growth chamber, usually with a complete nutrient solution containing 5 mM Ca (7). A low Ca (1 mM) nutrient solution was used in some experiments. Twice a week the used solution was discarded, and fresh solution was added through the pots to flush out accumulated salts. The solution level was maintained between changes by addition of distilled water to the reservoirs.

Pollen was collected daily, and when not used fresh was composited and stored at about 4 C in glass vials for later use. The pollen germination medium proposed by Brewbaker & Kwack (3), adjusted to pH 7.0 with NaOH, was used for germination of pollen in vitro. Different concentrations of Ca were added as $Ca(NO_3)_2$, and F^- was added as NaF. For each assay, 60 to 100 pollen grains were sown in a 0.005-ml standing drop of medium on a cover-slip. The cover-slips were placed on moist filter paper in petri dishes at room temperature, and germination counts were made after 2 hr.

To investigate the effects of HF fumigation of tomato plants on pollen germination and tube growth in vivo, the pollen tubes were observed by means of fluorescence after staining with aniline blue as described by Martin (5). Four indices of the pollen response were appraised: (i) the number of pollen grains visible on the stigma, estimated to the nearest 30 grains; (ii) the percent germination of the pollen grains present, estimated to the nearest 10% on the basis of the number of pollen tubes visible near the top of the style; (iii) the percent of the germinated pollen with tubes extending through the style; and (iv) the percent of the germinated pollen whose tubes had reached ovules. Early tomato fruit and seed development were evaluated under similar HF treatments for comparison with the pollen responses.

Plant samples were dried in a forced draft oven at 70 C and ground in a Wiley mill. We analyzed samples for F^- by slurrying with CaO, ashing, fusing with NaOH, isolating the F^- by perchloric acid distillation, and titrating with thorium nitrate (10, 11).

Duncan's multiple range test was used for statistical analysis of the data on pollen germination in vitro. Comparisons of the confidence intervals of the individual treatment means were used to determine the significant differences in the other experiments.

RESULTS.—*Pollen germination in media containing F^-.*—The response of pollen germination in vitro to F^- in the germination medium was evaluated with freshly collected tomato and cucumber pollen from plants grown with the standard nutrient solution. The germination media consisted of the basic medium with all combinations of 1.25, 2.5, and

TABLE 1. Percent germination of tomato and cucumber pollen in media containing various concentrations of Ca and F^-

Total Ca (mM)	Total F^- (mM) 0	1.3	2.6	5.3	10.5	15.8
Tomato[a]						
0	38 a					
1.25	40 a	42 a	42 a	32 a	13 b	1.5 b
2.50	46 a	47 a	40 a	44 a	16 b	4.4 b
3.75	43 a	41 a	44 a	42 a	31 a	0.2 b
Cucumber[a]						
0	65 a					
1.25	72 a	66 a	51 b	38 c	7 d	2.7 d
2.50	74 a	76 a	72 a	62 a	10 d	1.1 d
3.75	76 a	74 a	68 a	66 a	38 c	0.5 d

[a]For each plant species, means followed by the same letter are not significantly different at the 5% level. Based on Duncan's multiple range test; 15 assays/medium.

TABLE 2. Germination of tomato and cucumber pollen in vitro as influenced by HF fumigation of the plants, the Ca content of the nutrient solutions, and the presence or absence of Ca in the pollen germination medium

HF concentration ($\mu g\ F/m^3$)	Interval from starting HF treatment to pollen collection	Assays/ treatment	Standard nutrient solution				Low Ca nutrient solution			
			+Ca medium		-Ca medium		+Ca medium		-Ca medium	
			Control	HF	Control	HF	Control	HF	Control	HF
	days	*no.*	%	%	%	%	%	%	%	%
Cucumber										
10.2	22-50	50	68 a[a]	64 a	31 b	53 a	64 a	68 a	16 b	22 b
9.2	7	20					60 ab	71 a	45 b	55 ab
2.2	10-42	100	54 a	64 a	22 b	23 b	61 a	66 a	12 b	10 b
Tomato										
13.0	6-36	50	17 bc	26 ab	28 ab	24 ab	26 ab	14 c	35 a	18 bc
8.7	88	20	41 ns	45 ns	27 ns	34 ns				
7.9	55-125	90					9.3 a	5.0 b		
4.9	24-120	60	29 ns	23 ns						
4.6	4-39	55	20 ns	18 ns						

[a]Means in each experiment (on same line) followed by same letter are not significantly different at the 5% level; ns = no significant difference. Based on Duncan's multiple range test.

3.75 mM Ca and 0, 1.3, 2.6, 5.3, 10.5, and 15.8 mM F^-. Fifteen replicate assays were made with each type of pollen in each medium.

Germination of tomato pollen was almost completely inhibited in all media with 15.8 mM NaF (Table 1), and the tubes of the pollen grains that did germinate were stubby. Germination also was significantly reduced in the media containing 10.5 mM NaF, except at the highest Ca level. Some fairly large differences among treatments were not statistically significant because germination of the tomato pollen varied considerably within treatments.

The percent germination of the cucumber pollen was higher and less variable than for the tomato pollen. Cucumber pollen germination was significantly reduced at F^- levels as low as 2.6 mM in media with 1.25 mM Ca and at 10.5 mM F^- with the two highest levels of Ca. As with tomato pollen, inhibition of cucumber pollen germination was nearly complete in all media containing 15.8 mM F^-.

There was no reduction in germination of either tomato or cucumber pollen when the Ca was at least chemically equivalent to the F^- in the germination medium. Presumably, this is because of the low solubility of CaF_2 (0.016 g/liter at 18 C).

Germination of pollen from HF-fumigated plants.—Three experiments were conducted with cucumber and five with tomato in which pollen from HF-fumigated plants and comparable control plants was germinated in vitro (Table 2). Three of the experiments involved plants grown in both standard and low Ca nutrient solutions. In these and two other experiments, germination was tested in a medium with 1.25 mM Ca and in one without Ca. Pollen that had been stored for 8 to 23 weeks was used in two of the tomato experiments (at 7.9 and 4.6 $\mu g\ F/m^3$). Freshly collected pollen was used in the other experiments. In one experiment with each plant species, only a single set of assays was made. In the other experiments, the tests extended over several weeks; but since the results did not differ with time, only the over-all means for each treatment are shown in Table 2.

Little indication was found that pollen germination in vitro is affected by HF fumigation of the plants during pollen development. Cucumber pollen germination was related primarily to the Ca treatments. Germination generally was best in the medium with Ca, and this response was greatest with pollen from plants grown with low Ca nutrient solution. The one significant difference that occurred in the experiment when 9.2 $\mu g\ F/m^3$ was used also may be related to the Ca content of the pollen media, although a comparison between control and HF-treated plants also is involved.

Tomato pollen germination in vitro was not significantly affected by the Ca content of the germination media. The only differences found were associated with HF fumigations of tomato plants grown with low Ca nutrient solution. Germination of pollen from plants grown with solutions containing 1 mM Ca was significantly less when the plants were fumigated at either 7.9 or 13.0 $\mu g\ F/m^3$ than for comparable control plants. The 13.0 $\mu g\ F/m^3$ treatment had no apparent effects on pollen germination when the plants were grown in the solution containing 5 mM Ca; and no differences in germination were evident in the other tomato experiments, in which only the standard nutrient solution was used.

Responses of pollen in vivo to HF fumigation of plants.—Five experiments were conducted to evaluate the effects of HF fumigation of tomato plants on pollen germination and pollen tube growth in vivo

TABLE 3. Response of pollen tube growth in vivo to HF treatment of the plants

HF treatment (μg F/m^3)					
Pollen parent	Maternal parent	Pistils evaluated	Pollen on stigma	Germination	Pollen tubes reaching ovules[a]
		no.	*no.*	*%*	*%*
		Experiment A (self-pollination)			
C[b]	C	151	98 a[c]	62 a	20 a
9.8	9.8	171	24 b	28 b	9 b
		Experiment B			
C	C	42	190 a	76 a	27 a
C	7.9	24	100 c	50 c	15 bc
7.9	C	70	160 b	71 b	18 b
7.9	7.9	42	64 c	41 c	7 c
		Experiment C			
C	C	21	180 a	78 a	35 ns
C	5.0	29	73 c	50 bc	45 ns
7.9	C	25	120 b	60 b	47 ns
7.9	5.0	22	46 d	48 c	37 ns
		Experiment D			
C	C	12	170 a	84 a	52 a
5.0	5.0	12	110 b	63 b	32 b
		Experiment E			
C	C	60	77 a	69 a	37 a
C	4.2	60	49 b	38 b	22 b
4.5	C	60	92 a	65 a	44 a
4.5	4.2	60	38 b	38 b	10 b

[a] Percentage values for pollen reaching ovules are percent of germinated pollen.

[b] Control atmospheres (C) averaged less than 0.01 μg F/m^3.

[c] Within each experiment, means in the same column that are followed by the same letter are not significantly different at the 5% level. Based on confidence intervals of individual means.

(Table 3). The only significant difference found in pollen tube growth through the style was in experiment A. Therefore, that parameter was omitted from the table.

In experiment A, tomato plants growing in low Ca nutrient solution were allowed to self-pollinate. Flowers that opened from 27 to 53 days after the plants were placed in the treatment chambers were collected from 2 to 10 days after anthesis. The HF treatment averaged 9.8 μg F/m^3. Each criterion evaluated was significantly lower for the HF-treated plants than for the controls. Since no significant differences were found in comparisons of data for flowers collected at different intervals after pollination, the data for all collection intervals (2 to 10 days) were combined. Figure 1 shows typical pollen tube growth in pistils from control (A) and HF-treated plants (B).

Four experiments were conducted in which flowers on control and HF-treated tomato plants were manually pollinated with pollen from plants exposed to various HF and control treatments, in an effort to distinguish between the effects of HF on the pollen parent (donor) and on the maternal parent (recipient). The corollas and stamens were removed early on the day the flowers opened, and the selected pollen was applied to the stigma in the afternoon. Two to 11 days later, the pistils were collected for staining. Checks of unpollinated flowers showed that no self-pollination occurred prior to removal of the stamens.

Following experiment A, the same plants were retained under the same growing conditions and used for manual pollinations (experiment B). Pollen was accumulated from flowers on these plants and stored until used to pollinate flowers that opened during a 42-day period. The HF treatment averaged 7.9 μg F/m^3 during this experiment. The number of pollen retained on the stigma, the percent germination, and the percent of the pollen tubes reaching ovules all were significantly less for the HF-treated maternal parents regardless of the source of pollen. Significant reductions in the same three criteria resulted from the HF treatment of the pollen parents when the pollen was applied to control flowers, but not when applied to flowers of HF-treated plants.

Following an average storage period of 8 weeks, pollen collected during experiment B was used to pollinate flowers on plants grown with the standard nutrient solution and an HF treatment that averaged 5.0 μg F/m^3 (experiment C). Flowers opening between 4 and 49 days after the plants were placed in the HF treatment were manually pollinated. Significantly fewer pollen were retained on the stigma as a result of HF treatment of either the pollen parent or the maternal parent. Also, germination of pollen from either control or HF-treated plants was significantly lower on HF-treated than on control maternal plants. Pollen from HF-treated plants germinated less than control pollen on control maternal plants, but not on HF-treated maternal parents. No significant differences were found in pollen tubes reaching ovules.

In experiment D, 12 flowers each in the control and HF treatments of experiment C were pollinated with fresh pollen from the same respective treatments. Comparison of the data with those for experiment A shows similar results, but smaller differences occurred with the lower HF treatments in experiment D.

In the final experiment (E) of this series, the standard nutrient solution was used and the HF treatment of the maternal plants averaged 4.2 μg F/m^3. Two ages of pollen were used. Pollen composited from plants for which the HF treatment averaged 4.5 μg F/m^3 was stored for a mean period of 8 weeks and then used to pollinate flowers that opened from 17 to 38 days after the maternal plants were placed in the HF treatment. Fresh pollen was used to pollinate flowers on the same plants but opening between 41 to 58 days after the plants were placed in the atmospheric treatments. Thirty flowers in each treatment were pollinated with each age of pollen. The number of pollen on the stigma, the

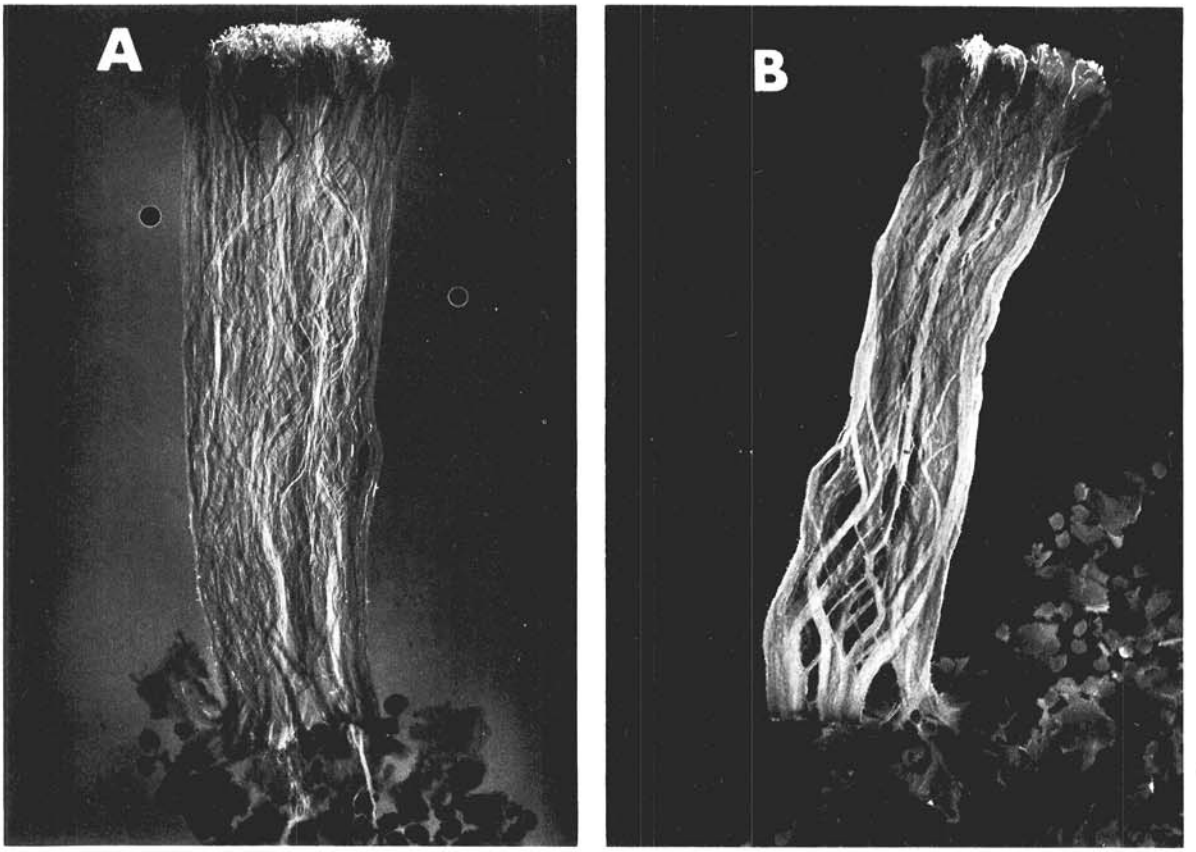

Fig. 1. Selected squash mounts of stained tomato pistils (× ca. 11) showing typical differences in amount of pollen retained on the stigma and pollen tube growth within the style. **A)** Control. **B)** HF-treated, 4.9 μg F/m^3. Upon staining with aniline blue, the callose within the pollen and pollen tubes fluoresces, under ultraviolet light, ca. 365 nm.

percent germination, and the percent of the pollen tubes reaching ovules all were significantly less for HF-treated maternal plants than for control maternal parents, regardless of the source of the pollen. There were no significant differences associated with HF treatment of the pollen parents. The fresh pollen gave consistently higher values than the stored pollen for all factors evaluated in experiment E, but the responses to HF were virtually the same for both types of pollen. Therefore, the data were combined for presentation in Table 3.

Effects of HF on tomato fruit and seed development.—Early development of tomato fruit was evaluated in experiments similar to those used for evaluation of pollen germination and tube growth in vivo. The only plants utilized in these experiments that were grown with low Ca nutrient solution were the pollen parents subjected to 7.9 μg F/m^3 and their controls. Flowers on plants growing in a control atmosphere were pollinated with (i) freshly collected pollen from plants growing at 4.5 μg F/m^3 and from comparable control plants; (ii) pollen that had been stored for a mean period of 4 weeks after collection from plants for which the HF treatment averaged 5.0 μg F/m^3; and (iii) pollen that had a mean storage period of 18 weeks after collection from plants for which the HF treatment averaged 7.9 μg F/m^3. Fifteen days after pollination, the fruit were picked, weighed, and the diameters measured; and seed development was determined. Seed development was rated 0 to 6 with respect to the number of seed forming in the fruit. All of the parameters were significantly less with pollen from the plants subjected to 7.9 μg F/m^3 than with control pollen (Table 4). Significant reductions in seed development and fruit diameter, but not in fruit weight, were also obtained when fresh pollen from plants grown at 4.5 μg F/m^3 was used. No significant differences in fruit or seed development resulted from pollination with stored pollen produced at 5.0 μg F/m^3.

In a similar experiment designed to include HF treatment of the maternal parents, flowers on tomato plants growing at 4.9 μg F/m^3 and comparable controls were pollinated with (i) freshly collected pollen from each treatment; (ii) stored (18 weeks) pollen from plants for which the HF treatment averaged 7.9 μg F/m^3; and (iii) stored (8 weeks) pollen from plants for which the HF treatment

TABLE 4. Tomato fruit and seed development on plants in control atmosphere 15 days after pollination with pollen from plants grown in various HF and control treatments

HF treatment of pollen parent (μg F/m^3)	Fruit evaluated	Fruit wt		Fruit diam		Seed development[a]	
		Control	HF	Control	HF	Control	HF
	no.	*g*	*g*	*mm*	*mm*		
4.4	76	9.48	7.62	25.9	22.9[b]	4.2	3.6[b]
5.0	31	7.10	7.84	19.2	19.9	2.6	2.5
7.9	53	5.90	3.66[b]	21.5	16.8[b]	3.7	2.9[b]

[a] Based on ratings of 0 to 6 (0, no seeds developing; 6, all seeds developing).
[b] Significantly different from the control at 5% level. Based on confidence intervals of individual means.

averaged 4.6 μg F/m^3. There were 21 flowers for each of the 12 treatment combinations, and the fruit were evaluated at 15 days as before. These cross-pollination studies showed effects on seed development, fruit diameter, and fruit weight in most of the comparisons between HF-treated and control maternal parents. However, the only significant effects associated with HF treatment of the pollen parents were reductions in seed development where stored pollen from plants grown at 7.9 μg F/m^3 and fresh pollen from plants grown at 4.9 μg F/m^3 were used on flowers of control plants (Table 5).

DISCUSSION.—The inhibitions of pollen germination, pollen tube growth, and early seed and fruit development by F^- provide a possible explanation for the reduced seed and fruit production by HF-fumigated tomato and bean plants observed previously (7, 8). Appreciable inhibition of pollen germination and pollen tube growth undoubtedly prevents some ovules from being fertilized, and consequently, fewer seeds are produced. In turn, fruit development is affected because of its dependence on auxin produced by the developing seeds (6).

A definite inverse relationship was found between the extent of the effects of F^- and the level of Ca in either the plant nutrient solution or the pollen germination medium. A similar relationship between F^- and Ca was noted in a previous study of tomato fruiting (7). The negative response of pollen germination to F^- in the germination media suggests that accumulation of F^- in the pistil could inhibit fertilization. However, because of the low solubility of CaF_2, little F^- will remain in solution if chemically equivalent amounts of dissolved Ca are present. Effects of HF on pollen germination and pollen tube growth in vivo were most readily produced when HF treatments were applied to the maternal parent plants. The inhibitions may have been due to accumulation of soluble F^- in the pistil; or, if soluble Ca is essential for good pollen germination and subsequent tube growth as indicated by Brewbaker & Kwack (3), precipitation of CaF_2 may bring about an inadequate supply of available Ca within the stigma or the surface fluids and thereby affect pollen germination and tube development.

HF fumigation of the pollen parents also caused reductions in pollen germination and tube growth, suggesting effects on pollen viability. Possibly F^- accumulated in the pollen, affecting it directly; or the F^- may have interfered with metabolism in the parent plants in some way, so that less viable pollen was produced. Such a response usually required rather high HF levels, and effects were more likely if the plants were growing in a low Ca nutrient solution.

Without question, the observed lower retention of pollen on the stigmas is a real response to the HF treatments. Directly or indirectly, F^- may cause failure of the pollen to germinate and/or produce enough tube development to hold the grains in place during the staining regime. Application of the pollen to the stigmas was uniform for all treatments, and no differences were noted in stain uptake by the pollen whether exposed to HF or not.

TABLE 5. Fruit and seed development 15 days after pollination in cross-pollination experiments involving various HF-treated and control plants

HF treatment (μg F/m^3)		Fruit wt (g)	Fruit diam (mm)	Seed development[a]
Pollen parent	Maternal parent			
		Stored pollen (18 weeks)		
C	C	5.74 a[b]	19.4 a	2.9 a
C	4.9	1.00 b	7.57 b	1.5 b
7.9	C	2.75 a	11.9 ab	1.7 b
7.9	4.9	0.87 b	6.36 b	1.0 b
		Stored pollen (8 weeks)		
C	C	7.66 a	23.5 a	3.3 a
C	4.9	2.36 bc	9.67 bc	1.6 bc
4.6	C	4.35 ab	16.2 ab	2.5 ab
4.6	4.9	0.83 c	6.21 c	1.0 c
		Fresh pollen		
C	C	9.62 a	26.3 a	4.1 a
C	4.9	3.86 ab	12.4 b	1.8 bc
4.9	C	6.95 ab	21.0 ab	3.1 b
4.9	4.9	3.34 b	12.6 b	1.6 c

[a] Based on ratings of 0 to 6 (0, no seeds developing; 6, all seeds developing).
[b] For each set of pollen, means in the same column that are followed by the same letter are not significantly different at the 5% level. Based on confidence intervals of individual means of 21 fruit/treatment.

A number of questions remain unresolved. Since F^- is not readily translocated between plant organs, a flower probably must be exposed directly to HF to be affected; but this was not established. Also, the length of the vulnerable period during which fertilization of a particular flower may be affected by exposure to HF was not determined since only long-term, continuous HF fumigations were used. Related to this is the unanswered question of whether an extremely high HF concentration for a very short period will have a greater effect than the same total dosage over perhaps 1 day or more.

The HF treatments that produced effects in this investigation were rather high in comparison to the levels of gaseous fluorides that are commonly encountered in the atmosphere. Therefore, such effects may not be a significant problem with tomatoes and cucumbers in the field. However, unpublished data by the authors have shown that for some other plants, e.g., corn, soybean, and strawberry, fruiting is more sensitive to HF, and the results reported here should be applicable to effects of F^- on plant fruiting in general.

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